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HEMOGLOBIN-BASED OXYGEN-CARRYING RESUSCITATION FLUIDS

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A. FOR YOUR REVIEW

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Currently, transfusion of donor blood is the conventional treatment for profound hemorrhagic shock (?ATLS). However, very significant problems are inherent in human blood transfusions. First, there is an increasing shortage of donors which becomes more severe due to the rising incidence of AIDS carriers in blood-donating populations (5). Second, red blood cells carry multiple antigenic determinants so that donor blood must be carefully typed and cross-matched before administration, a time-consuming and costly process. Third, blood has a limited "shelf-life" due to short storage viability, which at the present does not exceed one month. Fourth, blood storage and distribution is logistically-demanding because it must be stored and delivered at 4°C. Finally, blood can carry virally-transmitted diseases such as AIDS and hepatitis. Therefore, there is a profound clinical, economical and logistical demand for a non-antigenic, disease-free, easily transportable and long-term storable blood substitute.

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The hemoglobin tetramer has been the leading candidate for such a blood substitute because of its natural property to carry, deliver and release oxygen in a cooperative manner, its high saturation at ambient oxygen pressures, and its capacity to harbor as much as 1.39 ml of oxygen per gram protein. Indeed, three hemoglobin-based strategies are currently under development for use in patients with hemorrhagic shock: a) stroma-free hemoglobin (SFH), b) modified SFH, and c) liposome encapsulated hemoglobin (LEH).

~~Stroma-Free Hemoglobin~~

The rationale for the production of SFH is to avoid the

antigenic determinants of the red blood cell which are embedded solely within the erythrocyte membrane. However, early on in the development of SFH, it became apparent that purification of hemoglobin from human red blood cells did not produce a viable blood substitute. SFH failed to release sufficient amounts of oxygen into tissues due to its increased affinity to oxygen caused by the loss of intra-cellular 2,3-DPG (?p.1,12-14,19). Furthermore, due to the ~~significant~~ oncotic effect of the free hemoglobin solutions, only 6-8 g/dl of membrane-free hemoglobin could be ~~administered as a 1:1 volume~~ of blood replacement without risking volume overload (?p1.10). Also, the rapid dissociation of the hemoglobin tetramer to monomeric and dimeric forms precipitates renal clearance of SFH. Consequently, SFH has a very short half-life ($t_{\frac{1}{2}}$<6 hr, ?p.1,16) insufficient for the resuscitation of patients with hemorrhagic shock. Most importantly, SFH caused significant side effects such as nephrotoxicity (?) and vasoconstriction (?).

To eliminate some of these undesired characteristics, SFH has been chemically modified. For example, pyridoxine 5-phosphate was added to SFH to lower its affinity to oxygen so it would match that of whole blood (?). Also, polymerization (?) or cross-linking (?malcolm ref 19) of the α chains prevented the dissociation of SFH and therefore increased its half-life in the circulation and enabled the use of non-anemic solutions. A similar effect was obtained by the addition of long, linear strands of poly-ethylene glycol (PEG) which result in a high molecular weight protein not filtered through the kidneys (?#65). Animal studies with modified

SFH have been encouraging. For example, polymerized-pyridoxylated SFH at a concentration of 14 g/dl has been shown to support life after complete or near-complete exchange transfusion in baboons (146) and sheep (147). Also, diaspirin cross-linked hemoglobin was effective as whole blood and superior to crystalloid and colloid solutions in the treatment of hemorrhaged rats (jtrauma35:619).

Although animal tests with the various modifications of SFH have proven promising, human studies did not fare well with several clinical trials being prematurely terminated when side effects emerged. For example, in 1978, infusion of SFH into eight healthy volunteers produced transient but significant renal failure in spite of adequate hydration (?civetta21). Also, in the early 80's a study conducted in Germany using a carefully prepared and tested solution was discontinued following the development of severe renal failure in two healthy volunteers. In 1989, the FDA halted a clinical trial that attempted to test the safety of polymerized human hemoglobin in post-operative anemic but stable patients because of side effects such as shortness of breath and tightness in the chest (?business). Interestingly, in an earlier study the same product was reported to exert no side effects in six well hydrated healthy volunteers (?civetta22). More recently, a successful trial with modified bovine hemoglobin in 10 healthy volunteers in Guatemala has been reported (?). Nevertheless, a similar study in the US was abruptly halted for undisclosed reasons.

The encapsulation of the hemoglobin tetramer within a non-antigenic, synthetic phospholipid vesicle provides a logical

alternative for a resuscitative fluid. In theory, the phospholipid bilayer should protect the body from the toxic effects of free hemoglobin without interfering with oxygen transport and release. That is, the lipid membrane should reduce antigenicity and eliminate massive glomerular precipitation of dimeric and monomeric hemoglobin. Encapsulation of hemoglobin in a liposome should thus increase the circulation time and reduce the nephrotoxicity observed following the infusion of SFH. In addition, the concentration of hemoglobin into a reduced number of particles, compared to the free hemoglobin solutions, diminishes the osmotic activity of LEH and thus enables a higher hemoglobin concentration.

LEH was found to be an effective oxygen-carrier undergoing reversible cooperative oxygenation (27,28) with P_{50} and Hill constant values similar to that of whole blood. The kinetics of binding and release of oxygen to LEH were shown to be at least as rapid as for red blood cells (29). *In vivo*, initial safety studies with hydrogenated soy lecithin-based LEH (first generation LEH) identified several side effects such as tachycardia, hemoconcentration, leukopenia and thrombocytopenia associated with the liposome composition (30). Since lipid impurities and platelet activating factor (PAF) were implicated in the pathogenesis of these toxicities, an improved second generation LEH based on synthetic distearoyl phosphatidylcholine was developed (31), and the PAF antagonist BN 50739 was co-administered with LEH (4). The safer second generation LEH was then tested in a 50% isovolemic exchange transfusion rat model and found to exert superior hemodynamic and metabolic responses as compared to the standard

crystalloid solutions (32). Recently, the therapeutic efficacy of a newly produced third generation LEH was evaluated in a lethal rat model of hemorrhagic shock (35). This novel LEH preparation, which is a modification of the second generation LEH, consists of lyophilized powder LEH to be used as an "instant blood" at any prehospital civilian or combat arenas. This study demonstrated a remarkable salutary effect of LEH in treatment of hemorrhagic shock as the lyophilized preparation improved tissue oxygen tension , hemodynamics and survival (35). Also, lyophilized LEH has been shown to maintain stability and *in vitro* function characteristics for 3 months (36), and to have a similar circulation persistence as the liquid form (15-20 hr in mice, 37). Furthermore, lyophilized LEH maintains some physico-chemical properties required for use in the resuscitation of trauma victims such as half-life of 20 hrs, P_{50} of 20 mm Hg and a hill co-efficient of 2.8 (36).

While the potential clinical benefits of LEH are obvious, the yet unexplored safety issue with all LEH preparations is their impact on the function of the reticulo-endothelial system (RES). Recent studies utilizing radioactive labeling techniques have clearly demonstrated that LEH is predominantly cleared from circulation by the liver and spleen (grant47). Therefore, potential toxicities could arise from blockade or toxic ablation of the RES by LEH vesicles localized in liver and spleen macrophages. Indeed, the issue of LEH interaction with the RES has recently received much attention with several studies showing modulation of inflammatory reactions by LEH (langdale,alan,adhesion).

One of the major concerns in the development of all

hemoglobin-derived blood substitutes is the source of the hemoglobin used. To date, most laboratories are using outdated human or bovine hemoglobin. The first is subjected to many of the drawbacks associated with the collection, storage and transfusion of whole blood, mainly donor-dependent availability and the potential transmission of viral diseases. Bovine hemoglobin can transmit blood-borne diseases and being a foreign protein may elicit immune reactions. To address this issue, recombinant human hemoglobin was recently produced using an expression vector containing one gene encoding a mutant ß-globin with decrease oxygen affinity and one duplicated, tandemly fused α-globin gene (#108). Alternatively, transgenic pigs were reported to produce human hemoglobin as 10-15% of their total hemoglobin (science). Clearly, genetically engineered hemoglobin is the most attractive source for hemoglobin that can be incorporated into any delivery system. Also, such hemoglobin could theoretically possess all the desired characteristics of oxygen-carrying fluids.

Little is known on several most relevant issues related to the development and use of hemoglobin-based blood substitutes. For example, the immunogenecity of SFH, modified SFH and LEH and the immunological consequences of repeated administrations have not yet been addressed in depth. Also, the effect of these products on the various vascular beds and microvasculature is still obscure. In this respect, it is of utmost importance to elucidate the mechanisms of SFH and modified SFH-induced vasoconstriction. Furthermore, it is still unknown whether hemoglobin-derived oxygen-carrying solutions interfere with platelet function, coagulation

cascade, and typing and cross-matching of donor blood. Finally, safety concerns such as the effect of free hemoglobin on renal performance and the effect of LEH on RES function should be thoroughly investigated.

In summary, SFH and LEH are currently the most promising approaches in the search for an oxygen-carrying resuscitative fluid. Both strategies have shown efficacy in a variety of animal models of exchange transfusion and hemorrhagic shock while most clinical safety trials with SFH detected significant toxicities. Therefore, safety concerns seem to be the limiting factor in the clinical testing of hemoglobin-based blood substitutes. To overcome this problem, future research should be focused on understanding the mechanisms of SFH and LEH toxicity. Improved preparations, probably containing recombinant hemoglobin, should then be tested for clinical efficacy. Undoubtedly, both SFH and LEH will have a tremendous clinical and economical impact once these products become free of liabilities.